

Appl. No. : 10/584,338
Filing Date : January 9, 2007

AMENDMENTS TO THE CLAIMS

This listing of claims will replace all prior versions and listings of claims in the application.

LISTING OF CLAIMS

1-62. (Canceled)

63. (Original) A method of producing a cell population enriched in definitive endoderm cells, said method comprising the steps of:

differentiating cells in a population of pluripotent human cells so as to produce definitive endoderm cells, said definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom;

providing to said cell population a reagent which binds to a marker expressed in said definitive endoderm cells but which is not substantially expressed in other cell types present in said cell population; and

separating said definitive endoderm cells bound to said reagent from said other cell types present in said cell population, thereby producing a cell population enriched in definitive endoderm cells.

64. (Original) The method of claim 63, wherein the differentiating step further comprises obtaining a cell population comprising pluripotent human cells, providing said cell population with at least one growth factor of the TGF β superfamily in an amount sufficient to promote differentiation of said pluripotent cells to definitive endoderm cells, said definitive endoderm cells being multipotent cells that can differentiate into cells of the gut tube or organs derived therefrom, and allowing sufficient time for definitive endoderm cells to form, wherein said sufficient time for definitive endoderm cells to form has been determined by detecting the presence of definitive endoderm cells in said cell population.

65. (Currently amended) The method of claim [[63]]64, wherein detecting comprises detecting the expression of at least one marker selected from the group consisting of SOX17 and CXCR4 and at least one marker from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in cells of said cell population, wherein the expression of a marker selected from the

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group consisting of SOX17 and CXCR4 is greater than the expression of a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.

66. (Canceled)

67. (Currently amended) The method of claim [[63]]64, wherein detecting comprises detecting the expression of at least one marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 and at least one marker from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in cells of said cell population, wherein the expression of a marker selected from the group consisting of FGF17, VWF, CALCR, FOXQ1, CMKOR1 and CRIP1 is greater than the expression of a marker selected from the group consisting of OCT4, AFP, TM, SPARC and SOX7 in said definitive endoderm cells.

68. (Original) The method of claim 63, wherein at least about 95% of said cells are definitive endoderm cells.

69. (Original) The method of claim 63, wherein at least about 98% of said cells are definitive endoderm cells.

70. (Original) The method of claim 63, wherein said marker is CXCR4.

71. (Original) The method of claim 63, wherein said reagent is an antibody

72. (Original) The method of claim 71, wherein said antibody has affinity for CXCR4.

73. (Original) An enriched population of definitive endoderm cells produced by the method of claim 63.

74-75. (Canceled)

76. (New) A method of producing definitive endoderm cells, said method comprising:

obtaining a cell population comprising pluripotent human cells; and

providing said cell population with a TGF β superfamily growth factor and a Wnt-pathway activator, thereby generating in said cell population definitive endoderm cells expressing at least SOX17 and HNF3 β .

77. (New) The method of claim 76 further comprising removing TGF β superfamily growth factor from said cell population.

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78. (New) The method of claim 76, wherein at least 50% of said pluripotent cells differentiate into definitive endoderm cells.

79. (New) The method of claim 76, wherein said TGF β superfamily growth factor is selected from the group consisting of Nodal, activin A and activin B.

80. (New) The method of claim 76, wherein said TGF β superfamily growth factor is activin A.

81. (New) The method of claim 76, wherein said Wnt pathway activator is Wnt3a.

82. (New) The method of claim 76, wherein at least 10 ng/ml activin A is provided.

83. (New) The method of claim 76, wherein at least 100 ng/ml activin A is provided.

84. (New) The method of claim 76 further comprising the step of providing serum to said cell population in increasing concentrations.

85. (New) The method of claim 76, wherein said pluripotent cells comprise stem cells.

86. (New) The method of claim 76, wherein said pluripotent cells comprise embryonic stem cells.

87. (New) The method of claim 86, wherein said embryonic stem cells are derived from a tissue selected from the group consisting of the morula, the ICM of an embryo and the gonadal ridges of an embryo.